Proc. Natl. Acad. Sci. USA
Vol. 74, No. 7, pp. 2612–2613, July 1977
Chemistry

Magnetic properties and structure of oxyhemoglobin*

(hemoglobin/carbonmonoxyhemoglobin/iron electronic state/bond lengths/bond angles)

LINUS PAULING

Linus Pauling Institute of Science and Medicine, 2700 Sand Hill Road, Menlo Park, California 94025

Contributed by Linus Pauling, April 13, 1977

ABSTRACT **A review of the published evidence reinforces the conclusion reached by Pauling and Coryell in 1936 (*Proc. Natl. Acad. Sci. USA* 22, 210–216) that oxyhemoglobin in blood or in solution at 20° has zero magnetic moment and fails to support a recent contradictory suggestion based on magnetic susceptibility of frozen samples at temperatures below 250 K. Predicted values of bond lengths and bond angles for carbonmonoxyhemoglobin and oxyhemoglobin are given.**

The problem of the electronic structure of hemoglobin and its derivatives and in particular of the nature of the bond between the iron atoms and the attached oxygen molecules in oxyhemoglobin remains only partially solved and continues to attract the attention of researchers. A basic contribution was made in 1936 when it was reported that oxyhemoglobin and carbonmonoxyhemoglobin have zero magnetic moment and hemoglobin has a magnetic moment corresponding to four unpaired electrons with parallel spins for each heme iron atom (1). This observation, which permitted an illuminating discussion of the electronic structure of hemoglobin and its derivatives to be presented (1), has been accepted for 40 years. A study has recently been published, however, that contradicts the earlier result; the investigators concluded that oxyhemoglobin was shown by their observations not to be diamagnetic above 50 K, but instead to have the magnetic moment corresponding to two parallel electron spins for each FeO_2 group (2). The structural interpretation given this property is that one unpaired electron is to be assigned to the O—O bond and the other to the Fe—O bond and that there is a weak antiferromagnetic coupling between their spins. It is clear that the contradiction between the two reported experimental observations needs to be resolved.

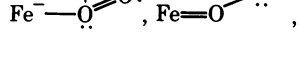
A very simple technique was used in the 1936 study. A glass tube about 18 mm in diameter was divided into two compartments by a glass septum. The lower compartment was filled with water and the upper one with defibrinated cow blood or a solution of hemoglobin or one of its derivatives obtained by hemolyzing the blood and separating the stromata emulsions by centrifugation. The tube was suspended from one arm of an analytical balance, with the septum between the pole pieces of an electromagnet, and the differences Δw in apparent weight for magnetic fields 0 and 7640 or 8830 gauss (Δw referred to 7640 gauss) were measured. The apparatus, which was in an air thermostat at 20°, was calibrated with the measured value of −49.59 mg for air-saturated water against air with use of the known values of their magnetic susceptibility. The standard deviation of the mean of values of Δw was about 0.1 mg. The average value of Δw for two samples of blood saturated with carbon monoxide and two samples of carbonmonoxyhemoglobin solution was −0.65 mg, in excellent agreement with the value of −0.58 mg calculated for zero magnetic moment with correction for differences in water content and dissolved air and for diamagnetism of the globin, and in pronounced disagreement with the value +1.52 mg for two unpaired electrons per heme. The values of Δw for two samples of oxygenated blood and two samples of oxyhemoglobin solution were found to differ from the values for the corresponding carbon monoxide derivatives by +0.13, +0.27, +0.02, and +0.04 mg, respectively. The calculated differences for two unpaired electrons per HbO_2, 2.03, 2.07, 3.74, and 4.11 mg, respectively, are so different as to show conclusively that in solution at 20° oxyhemoglobin, like carbonmonoxyhemoglobin, does not have two unpaired electrons per heme. This conclusion was verified for cow oxyhemoglobin by Coryell *et al.* (3) and for horse, sheep, and human oxyhemoglobin by Taylor and Coryell (4).

The recent study of human carbonmonoxyhemoglobin and oxyhemoglobin (2) was made on samples that were frozen at liquid-helium temperature. The magnetic susceptibility was measured through the range 30–250 K and the values for oxyhemoglobin were fitted by a computer to an equation based on the assumption of equilibrium between a singlet state for FeO_2 with magnetic moment zero and a triplet state with magnetic moment corresponding to two parallel electron spins. There were three adjustable parameters as follows: an additive term to the susceptibility in order to extrapolate at infinite temperatures to the observed value for carbonmonoxyhemoglobin, a term proportional to T^{-1}, attributed to dissolved oxygen and ferrihemoglobin impurity, and the singlet–triplet separation. The value of the singlet–triplet separation, 1.72 kJ mol^{-1} (referred to heme), is far smaller than would be expected for two electrons as close together as the iron atom and the oxygen molecule in the FeO_2 group. I think that when the oxyhemoglobin solution was frozen in liquid helium most of the water separated as nearly pure ice, leaving a largely dehydrated oxyhemoglobin phase, which may be metastable at temperatures below 40 K and partially dissociated at higher temperatures. The maximum molar susceptibility attributed by the investigators to FeO_2 was 2900×10^{-6} c.g.s.u. at 125 K. This value could result from about 10% dissociation of the oxyhemoglobin to oxygen and high-spin hemoglobin or from about 40% dissociation to oxygen and a form of hemoglobin with zero magnetic moment. Whatever the explanation of the reported magnetic properties of frozen solutions of oxyhemoglobin, it is not justified to draw from these experiments any conclusions about oxyhemoglobin in blood or in solution at room or body temperature.

In our first paper on magnetic properties of hemoglobin Coryell and I (1) suggested that carbonmonoxyhemoglobin and oxyhemoglobin had the resonating structures represented by the valence-bond structure

$$Fe^{-}—C{\equiv}O:^{+},\ Fe{=}C{=}\ddot{O}:,$$

and



* Publication no. 86 from the Linus Pauling Institute of Science and Medicine.

respectively. Later the suggestion was made that the structure $Fe^{+}{\equiv}C{-}\ddot{O}:^{-}$ might also make a contribution for carbonmonoxyhemoglobin (5). A recent discussion of the structure of high-covalence compounds of transition metals makes it possible now to refine these suggestions (6, 7). With the assumption that the three structures shown above contribute equally, the expected interatomic distances in carbonmonoxyhemoglobin are 175 pm for iron–carbon and 119 pm for carbon–oxygen. The bond angle at the carbon atom should be 180°, but the axis for the FeCO group may deviate considerably from the perpendicular to the plane of the four heme nitrogen atoms (7). The expected distances in oxyhemoglobin are 172 pm for iron–oxygen and 127 pm for oxygen–oxygen, and the expected bond angle is 114°, the value for a first-row atom forming a single bond and a double bond.

Note Added in Proof. Recent *ab initio* quantum mechanical calculations on models for oxyhemoglobin by Olafson and Goddard (8) have led to the conclusions that the triplet state of the FeO_2 group lies about 34 kJ mol^{-1} above the singlet state and that the FeOO bond angle is 119°.

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

1. Pauling, L. & Coryell, C. D. (1936) *Proc. Natl. Acad. Sci. USA* **22**, 210–216.
2. Cerdonio, M., Congiu-Castellano, A., Mogno, F., Pispisa, B., Romani, G. L. & Vitale, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 398–400.
3. Coryell, C. D., Pauling, L. & Dodson, R. W. (1939) *J. Phys. Chem.* **43**, 825–839.
4. Taylor, D. S. & Coryell, C. D. (1938) *J. Am. Chem. Soc.* **61**, 1263–1268.
5. Pauling, L. (1964) *Nature* **203**, 182–183.
6. Pauling, L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3799–3801.
7. Pauling, L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4200–4202.
8. Olafson, B. D. & Goddard, W. A., III (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1315–1319.